Phytochemical and Biological Studies on *Terminalia muelleri* Family Combretaceae

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By

Nouran Mohammed Mohammed Fahmy

Bachelor of Pharmaceutical Sciences,

Faculty of Pharmacy, Ain Shams University, 2011

Under the Supervision of

Abdel-Nasser B. Singab Ph. D.

Professor of Pharmacognosy

Dean of Faculty of Pharmacy

Ain Shams University

Eman Mohamed Kamal Ph. D.

Lecturer of Pharmacognosy

Faculty of Pharmacy

Ain Shams University

Department of Pharmacognosy
Faculty of Pharmacy
Ain Shams University
Abbasia, Cairo, Egypt
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Protective effect of *Terminalia muelleri* against carbon tetrachloride-induced hepato and nephro-toxicity in mice and characterization of its bioactive constituents

Abstract

Context: The genus *Terminalia* are used in folk medicine for the treatment of various disease.

Objective: To investigate the hepato and nephro protective activity of a polyphenol-rich fraction (TMEF) obtained from *Terminalia muelleri* Benth. (Combretaceae) against CCl_4-induced toxicity in mice.

Materials and methods: TMEF was administered (100, 200, and 400 mg/kg/day) for 5 days. CCl_4 was administered at the end of the experiment. Hepatic and renal biomarkers were measured in the serum. Glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) were estimated in the liver and kidney tissues. The active constituents of TMEF were identified by HPLC–PDA–ESI/MS/MS.

Results: TMEF is rich in ellagitannins, galloyl esters, phenolic acids and flavone-*C*-glucosides. TMEF pretreatment significantly ($P < 0.001$) inhibited the CCl_4-induced increase in ALT (17, 43, and 53%), AST (20, 46, and 58%), ALP (20, 48, and 56%), LDH (21, 47, and 58%), hepatic MDA (23, 49, and 54%), renal MDA (22, 35, and 52%), creatinine (48, 66, and 91%), uric acid (16, 34, and 59%), urea (22, 39, and 59%), and cholesterol (20, 27, and 46%). Furthermore, TMEF administration significantly ($P < 0.001$) increased hepatic GSH (15, 51, and 79%), renal GSH (23, 45, and 73%), hepatic SOD (9, 52, and 95%), renal SOD (39, 66, and 85%) and protein levels (17, 24, and 29%) at the tested doses of TMEF, respectively. Pretreatment with TMEF preserved the hepatic architecture and protected from ballooning degeneration, liver necrosis, renal inflammation and degeneration of the kidney tubules.

Conclusion: TMEF has a marked hepato-nephro protective effect.

Keywords: Hepatoprotective; antioxidant; nephroprotective; ellagitannins; flavone-*C*-glucosides; lipid peroxidation; glutathione

Poster presented in the 17th Congress of the Association of Pharmacy Colleges in the Arab World, held in Faculty of Pharmacy ASU, Cairo, Egypt. October, 2014

Phenolic profile and antioxidant activity of *Terminalia muelleri* Benth. leaf extract

Abstract

The chemical constituents of the ethanol-soluble fraction, obtained from the 80% methanol extract of *Terminalia muelleri* Benth. leaves, were characterized using the HPLC–PDA–ESI/MS/MS technique. A total of 19 compounds were identified including, ellagitannins, galloyl esters, phenolic acids and *C*-glycosidic flavonoids. The ethanol-soluble fraction of *T. muelleri* showed a potent radical-scavenging activity using the DPPH· (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay (IC_{50} value = 2.7 μg/ml, while the standard ascorbic IC_{50} value = 10.5 μg/ml).

Keywords: Ellagitannins; galloyl esters; DPPH; ascorbic acid.

CONTENTS

LIST OF ABBREVIATIONS

AChE	acetylcholinesterase
ACP	acid phosphatase
ALP	alkaline phosphatase
ALT	alanine aminotranferase
ANOVA	analysis of variance
APT	attached proton test
AST	aspartate aminotransferase
brd	broad doublet
bw	body weight
CC	column chromatography
CCl_4	carbon tetrachloride
CD_3OD	deutrated methanol-d_4
CH_2Cl_2	dichloromethane
cm	centimeter
COLO	human colon cancer cell lines
COSY	correlation spectroscopy
COX-1/ COX-2	cyclooxygenase enzyme 1/ 2
d	doublet
dil	dilute
dl	deciliter
DMSO-d_6	deutrated dimethylsulfoxide-d_6
$DPPH^{\bullet}$	2,2-diphenyl-1-picrylhydrazyl radical
ELISA	Enzyme linked immunosorbent assay
ESI	electrospray ionization
EtOAc	ethyl acetate
EtOH	ethanol
FBS	fetal bovine serum
g	gram
GSH	glutathione
h	hour/ hours
HbA_1c	glycated hemoglobin
HCT	human colorectal carcinoma cell lines
HDL	high density lipoprotein
HepG2	human hepatocellular liver carcinoma cell line
HHDP	hexahydroxydiphenic acid
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple bond correlation
HPLC	high performance liquid chromatography
HRESIMS	high resolution electrospray ionization mass spectrometry
HSQC	heteronuclear single-quantum correlation
Hz	hertz
i.p	intraperitoneal
IC_{50}	inhibitory concentration by 50%.
ID	internal diameter
IL-1β	interleukin-1β
IL-6	interleukin-6
***J* value**	coupling constant

kg	kilogram
l	liter
LDH	lactate dehydrogenase
LDL	low density lipoprotein
m	meter
m/z	mass to charge ratio
MBC	minimum bactericidal concentration
MDA	malondialdehyde
MDA-MB-231	m.d. anderson-metastatic breast cancer cell lines
MeOH	methanol
mg	milligram
MIC	minimum inhibitory concentration
min	minute/ minutes
ml	milliliter
mM	millimole
mm	millimeter
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	mass spectrometry
nm	nanometer
NMR	nuclear magnetic resonance
PC	paper chromatography
PDA	photodiode array
Pet. ether	petroleum ether
PGE_2	prostaglandin E_2
PPARα/ PPARγ	peroxisome proliferator-activated receptor alpha/ gamma
ppm	part per million
PTEF	polytetrafloroethylene teflon
ROS	reactive oxygen species
rpm	rotation per minute
RPMI	roswell park memorial institute medium
s	singlet
SEM	standard error of mean
SOD	superoxide dismutase
STZ	streptozotocin
T.	*Terminalia*
TLC	thin layer chromatography
TMEF	ethanol-soluble fraction of *Terminalia muelleri* leaf extract
TMS	tetramethylsilane
TNF-α	tumor necrosis factor alpha
t_R	retention time
UV	ultraviolet
VLDL	very low density lipoprotein
α	alpha
***δ* value**	chemical shift (ppm)
µg	microgram
µM	micromole

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